REPORT DOCUMENTATION PAGE

OMB No. 0704-0188

AD-A267 372

2 REPORT DATE	3 REPORT TYPE AND DATES COVERED
	ANNUAL 1 Jun 92 TO 31 May 93

4 TITLE AND SUBTITLE

(FY91 AASERT), RESEARCH TRAINING OF THE EFFECTS OF TOXIC SUBSTANCES ON THE LUNGS

5 FUNDING NUMBERS

F49620-92-J-0325
61103D
3484
S4

6 AUTHOR(S)

Dr Mark L. Witten

7 PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dept of Pediatric & Physiology
University of Arizona
1501 N. Campbell Avenue
Tucson, AZ 85724

8 PERFORMING ORGANIZATION REPORT NUMBER

9 SPONSORING MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR/NL
110 Duncan Avenue, Suite B115
Bolling AFB DC 20332-0001

Dr Kozumbo

10 SPONSORING MONITORING AGENCY REPORT NUMBER

DTIC
ELECTE
AUG 04 1993
S A D

11 SUPPLEMENTARY NOTES

93-17267

12a DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited

12b DISTRIBUTION CODE

13 ABSTRACT (Maximum 200 words)

Allison M. Hays is the graduate student supported by the AASERT grant and Jason T. Figueroa was the high school student supported by the same grant. Jason was supported by the AASERT grant during the Summer of 1992. He worked on two research projects during this time period. The first project concerned the effects of JP-8 jet fuel exposure on substance P receptors, specifically the NK1 receptor, in rats' lungs. This project encountered technical difficulties in determining the sensitivity of the assay used in quantifying the amount of NK1 receptors. Consequently, we did not attempt ot publish this data. Jason also worked on our acute smoke exposure project which is an established model of Acute Respiratory Distress Syndrome. His work with this project resulted in an abstract which he presented at the Experimental Biology '93 meeting in New Orleans. Jason was awarded a Flinn Foundation scholarship for this Fall to attend the Univ of Arizona and decided to terminate his association with the AASERT training grant. I have replace Jason with Brian Tollinger, a student who has just been accepted into the doctoral program in the College of Pharmacy. Brian is investigating the possibility of entering a combined Ph.D. in Toxicology- Pharm. D. program. Brian has worked in my laboratory for

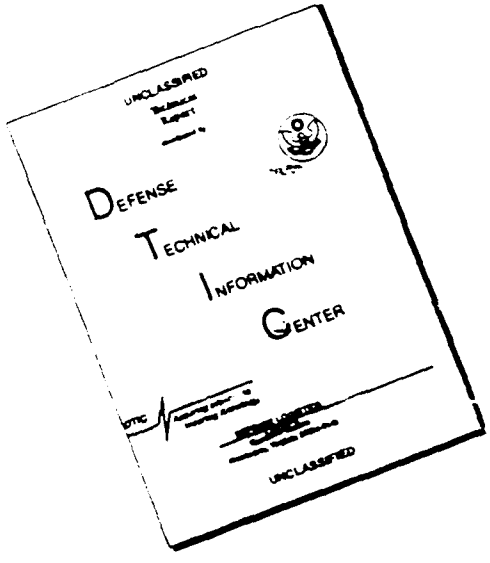
14 SUBJECT TERMS	15. NUMBER OF PAGES
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(UL)

NSN 7540-01-280-5500

Standard Form 298 (Rev 2-89)
Prescribed by ANSI Std Z39-18
298-102

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

FIRST YEAR SUMMARY FOR AASERT GRANT

ENTITLED

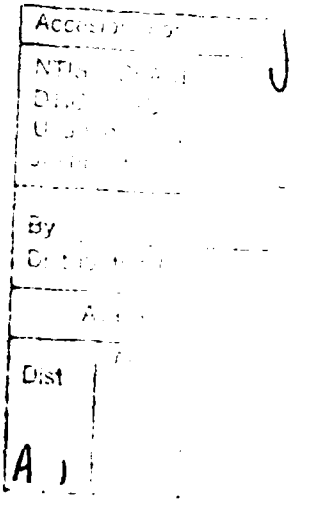
RESEARCH TRAINING OF THE EFFECTS OF TOXIC SUBSTANCES ON THE LUNGS

Mark L. Witten, Ph.D. Principal Investigator

Department of Pediatrics

Arizona Health Sciences Center

Tucson, Arizona



Submitted to-
Life and Environmental Sciences Directorate
U.S. Air Force Office of Scientific Research
Bolling Air Force Base, DC 20332-6448

25 JUN 1993

Over-all Progress of Grant

Allison M. Hays is the graduate student supported by the AASERT grant and Jason T. Figueroa was the high school student supported by the same grant. Jason was supported by the AASERT grant during the Summer of 1992. He worked on two research projects during this time period. The first project concerned the effects of JP-8 jet fuel exposure on substance P receptors, specifically the NK1 receptor, in rats' lungs. This project encountered technical difficulties in determining the sensitivity of the assay used in quantifying the amount of NK1 receptors. Consequently, we did not attempt to publish this data. Jason also worked on our acute smoke exposure project which is an established model of Acute Respiratory Distress Syndrome. His work with this project resulted in an abstract which he presented at the Experimental Biology '93 meeting in New Orleans. Jason was awarded a Flinn Foundation scholarship for this Fall to attend the University of Arizona and decided to terminate his association with the AASERT training grant. I have replaced Jason with Brian Tollinger, a student who has just been accepted into the doctoral program in the College of Pharmacy. Brian is investigating the possibility of entering a combined Ph.D. in Toxicology- Pharm. D. program. Brian has worked in my laboratory for the past year. His research project is to develop an assay for determining neutral endopeptidase concentrations in rat lung lavage and tissue samples after chronic exposure to JP-8 jet fuel using high pressure liquid chromatography.

Allison has worked on the JP-8 jet fuel inhalation toxicology project for the past two years under my direction and that of Dr. John K. Pfaff, a Navy physician who has conducted a post-doctoral Fellowship in my laboratory. Dr. Pfaff has been assigned duty at the Portsmouth Naval Hospital starting in July of 1993. Allison will assume Dr. Pfaff's duties on the JP-8 jet fuel inhalation toxicology project. Allison has also been heavily involved in our acute smoke exposure model of Acute Respiratory Distress Syndrome. This work resulted in an abstract that she presented at the Experimental Biology '93 meeting in New Orleans. Allison is doing well in her course work in preparation for entering the Doctoral program in the Department of Anatomy. However, she may transfer to the Department of Pharmacology/Toxicology doctoral program because she is concerned about future employment possibilities with a degree in Anatomy -vs- a degree in Toxicology. There is no doubt that a

Toxicology degree has many more employment possibilities than a degree in Anatomy at this point in time.

Plans for Year 2 of the Grant

Allison will take over the daily management of the Air Force JP-8 jet fuel project with Brian's assistance. Both Allison and Brian are intelligent students and I expect them to maintain their high level of performance in their coursework. Both will continue their individual research projects on the jet fuel project and I expect both of them to present their research at the Experimental Biology '94 meeting in Anaheim, California.

2938

PULMONARY NEUTROPHIL SEQUESTRATION AFTER INTESTINAL ISCHEMIA-REPERFUSION. M. Tuhy, B. Gewertz. University of Chicago, Chicago, Illinois 60637

Intestinal ischemia reperfusion is a known precipitant of acute lung injury. Neutrophils (PMN) and free radicals from PMN are critical to both processes. This study was designed to determine if the infiltration of PMN from intestinal reperfusate could attenuate lung PMN accumulation and injury. Four groups of rats (n=5 each) were subjected to 30 or 45 min of complete ischemia (superior mesenteric artery occlusion and collateral ligation). The SMA was then reperfused for 30 min with or without a leukocyte filter which removes 99+% PMN. Shams (n=3) underwent identical preparation without ischemia or filter (ischemic time = 0). After 30 min of reperfusion, lung tissue was assessed for PMN accumulation (myeloperoxidase levels) and alveolar capillary exit (I-125 albumin). Filtering reperfusion PMN reduced lung PMN accumulation at 30 min after reperfusion in rats suffering both 30 and 45 min of ischemia. In contrast, there was no increase in lung microvascular permeability at the same post-reperfusion interval.

Lung MPO (ΔA/min/gm)

	Ischemic Time		
	0 min	30 min	45 min
No Filter	32.2 ± 3.5	71.7 ± 7.8*/†	136.5 ± 7.8*/†
Filter		40.6 ± 10.8	69.8 ± 11.9*

* p<0.05 from 0
† p<0.05 from Filter

These data show that lung PMN accumulation occurs in the early period after intestinal reperfusion and predates functional evidence of lung injury. While a cause and effect relationship remains unproved, the known deleterious effects of PMN activation suggests that reperfusate filtration may have clinical application in decreasing distant organ dysfunction after intestinal ischemia.

Supported by NIH Surgical Scientist Training Grant)

ENVIRONMENTAL PATHOLOGY AND AIR POLLUTANTS (2939-2942)

2939

MECHANISMS OF SMOKELESS TOBACCO-INDUCED INCREASE IN MICROVASCULAR PERMEABILITY IN VIVO. I. Rubinstein, X.-p. Gao, J.M. Conlon and J.K. Vishwanatha. Univ. of NE Medical Center and Creighton University, Omaha, NE 68198.

The purpose of this study was to determine whether smokeless tobacco (SE) increases vascular permeability in the hamster cheek pouch, and whether these effects are mediated by local generation and release of bradykinin (BK), coupled with a decrease in tissue angiotensin I-converting enzyme (ACE) activity, which cleaves BK. Using intravital microscopy, we found that ST extract induced a significant ($p < 0.05$) concentration-dependent increase in leaky site formation and clearance of fluorescein isothiocyanate dextran (m.w. = 70,000 daltons) in the cheek pouch. These effects where significantly attenuated by two selective bradykinin B_2 receptors antagonists, Hoe 140 and NPC 17647. Suffusion of ST extract was also associated with a significant increase in BK concentration in the suffusate, and with a significant decrease in cheek pouch ACE activity. Levels of ACE protein and mRNA in the cheek pouch were not altered during suffusion of ST extract. We conclude that ST extract increases microvascular permeability in the hamster cheek pouch by local generation and release of BK, and by decreasing tissue ACE activity leading to potentiation of BK-induced responses.

2940

U75412E PRETREATMENT BEFORE ACUTE SMOKE EXPOSURE CAUSES A LARGE INCREASE IN LUNG PROSTACYCLIN CONCENTRATIONS. A.M. Hays, R.C. Lantz, M. Vermeulen, G. Chen, M.L. Witten. Steele Memorial Children's Research Center, University of Arizona, Tucson, AZ and Harvard Medical School, Boston, MA.

We utilized a rabbit model to analyze the effect of the lazaroid, U75412E, on its ability to attenuate severe lung injury induced by acute smoke exposure. The acute smoke insult consisted of 60 tidal volume breaths of diesel fuel-polycarbonate plastic smoke administered in 8-9 min. There were four groups of rabbits: Three hour smoke-exposed rabbits (THSE, N=8), Three hour sham smoke-exposed rabbits (THSS, N=6), Short (3-4 min) smoke-exposed rabbits (SSE, N=5), and U75412E pretreated smoke-exposed (USE, N=7). The lungs were removed immediately after the experiment and bronchoalveolar lavage (BAL) was performed with normal sterile saline. BAL fluid was analyzed for 6-keto-PGF1alpha, the stable metabolite of prostacyclin. The 6-keto-PGF1alpha concentrations (pg/ml BAL fluid) were the following:

THSE	506 (32)
THSS	307 (57)
SSE	142 (25)
USE	1495 (107)*

* $p < 0.05$.

The USE rabbits lived for six hours after the smoke insult before they were killed for cell culture studies. Perhaps, the increased lung prostacyclin production in the USE rabbits contributes to an attenuation of the smoke-induced lung injury by modulation of the airway bronchoconstrictive response to smoke. Supported by Upjohn.

2941

DOES LEAD PLAY A ROLE IN THE PATHOGENESIS OF METABOLIC BONE DISEASE? A HYPOTHESIS. H. Spencer, V. O'Sullivan and S. Sontag. Metabolic Research, V. A. Hospital, Hines, IL 60141.

We reported previously that 89% of patients with Paget's disease of bone gave a history of occupational exposure to lead (J. Lab. Clin. Med., 120:798-800, 1992). Bone is the prime target organ of the deposition and long-term storage of lead. The question arose whether lead may also play a role in metabolic bone diseases other than Paget's disease. That this may apply to hyperparathyroidism was considered. Reason: others have shown that lead interferes with the utilization of vitamin D and induces vitamin D deficiency which would lead to decreased intestinal absorption of calcium, a low calcium status and subsequent parathyroid stimulation. The histories of 4 patients with proven hyperparathyroidism revealed that they were occupationally or environmentally exposed to lead for many years. A 5th patient, on whom no occupational history was obtained, may have been exposed to lead as he had both Paget's disease and hyperparathyroidism. We are extending our series at present. These preliminary observations should stimulate others to investigate whether lead is one of the factors which may play a role in the pathogenesis of the metabolic bone disease hyperparathyroidism.

2942

ADVERSE HEALTH EFFECTS ASSOCIATED WITH CHROMIUM EXPOSURE IN WORKERS EMPLOYED IN A CHROMATE INDUSTRY. D.M. Backyavathy and N.V. Nanda Kumar (SPON: M.K.D. Paga.a). P.G. Department of Zoology, Voorhees College, Madras University, Vellore 632 001 and Department of Zoology, Sri Venkateswara University, Tirupati 517 502, INDIA

Adverse health effects of Chromate in industrial workers exposed to occupational environment have been reported. Four hundred workers of different age groups employed in a chromate factory, showed occurrence of skin diseases like chrome-hold ulcer(28.75%), industrial dermatitis(18.75%), acid burns(9.5%), injuries (13.75%) and respiratory diseases like bronchitis (77.5%), acute pharyngytis (88.25%), fibrositis (2.5%), pleurisy (3%) and also nasal irritation and nasal perforation (18.25%) with high relative risk factor. The above adverse health effects are associated with occupational environment of the chromate factory. Total chromium content in Urine and Blood in workers ranged from 6.2-17.5 µg/L urine and 2.4-12.7 µg/100 G blood respectively.

2360

Acrolein and other aldehydes present in tobacco smoke induce activation/inactivation of protein kinase C – protection by thiol agents.
D. Jantzen, U. Gundimeda, Z. H. Chen, and R. Gopalakrishna.
Dept. Pharmacol. & Nutr., USC Sch. of Med., Los Angeles, CA 90033

Acrolein, formaldehyde, and acetaldehyde are toxic agents present in tobacco smoke and in polluted air. These aldehydes induce pathobiological effects in lungs including tumor promotion. Since protein kinase C (PKC) is a receptor for tumor promoters, we have determined whether these aldehydes could influence PKC. In the bronchial epithelial cells and NRK cells treated with acrolein (0.5 to 5 μM) a rapid (min) 2- to 2.5-fold increase in PKC activity without any change in subcellular distribution was observed. However, prolonged treatment with acrolein resulted in an inactivation of PKC. These changes were also induced in purified PKC by direct exposure to acrolein. Both formaldehyde and acetaldehyde also induced similar cellular changes in PKC at much higher concentrations (>100 μM). N-acetylcysteine, L-cysteine, and a cell-permeable analogue of glutathione all protected PKC from the aldehyde-induced modifications of PKC in in intact cells. Thus, acrolein and other aldehydes induce pathobiological effects and tumor promotion in lungs, in part, by inducing activation/inactivation of PKC. Nonetheless, they differed from other tumor promoters (phorbol esters, oxidants) in that these aldehydes did not induce a cytosol to membrane translocation of PKC.
Supported by Grant RT388 TRDRP, University of California

2361

INCREASING SMOKE EXPOSURE PRODUCES [illegible] BY AIRWAYS NOT ALVEOLAR EDEMA WITHOUT LIPID PEROXIDATION.
[illegible], [illegible] Campbell, [illegible] Lalonde, R. Demling. Beth Israel Hospital, 330 Brookline Avenue, Boston, MA [illegible].

We determined the effect of a graded [illegible] in smoke exposure on lung physiologic response and oxidant induced lipid peroxidation (L.P.). [illegible] sheep were given 12 breaths of [illegible] cotton toweling smoke at a tidal volume (VT) of [illegible] ml, [illegible] ml, [illegible] ml/kg. Each breath contained particles of mean diameter of [illegible]. Animals were monitored unanesthetized for [illegible] hours. Lung (L.P.) was measured by [illegible] and alveolar malondialdehyde (MDA) and antioxidant activity as catalase and reduced glutathione (GSH). Data was compared to [illegible] controls. Histology revealed no changes with [illegible] ml/kg, but marked airways edema with the higher V_T. Alveolar edema was seen only with 20ml/kg. There was no decrease in catalase or GSH activity.

RESULTS: Mean±SD	PaO2/ FIO2	COHgb	LUNG WATER	LUNG MDA nMol/g TISSUE	MUCOSA
CONTROL	520±21	2±1	72±3%	[illegible]	12±3
SMOKE(10ml)	350±18*	26±3	74±3%	180±25	9±2
SMOKE(20ml)	260±55*	46±3	84±3%	185±32	10±2

*=significantly different from controls p<0.05

Correlation of impaired oxygenation with lung water was r^2=.3. We can conclude that: 1)increasing the area of smoke exposure increases lung dysfunction, 2)airways closure, not alveolar edema, is the cause of the lung dysfunction, and 3)lipid peroxidation is not an etiologic factor.

2362

U75412E PRETREATMENT BEFORE ACUTE SMOKE EXPOSURE INCREASES BAL VITAMIN E LEVELS.
J.T. Figueroa, D.C. Liebler, A.M. Hays, R.C. Lantz, M. Vermeulen, G. Chen, M.L. Witten. Steele Memorial Children's Research Center and Center for Toxicology, University of Arizona, Tucson, AZ and Harvard Medical School, Boston, MA.

We utilized a rabbit model to analyze the effect of the lazaroid, U75412E, on its ability to attenuate severe lung injury induced by acute smoke exposure. The acute smoke insult consisted of 60 tidal volume breaths of diesel fuel-polycarbonate plastic smoke administered in 8-9 min. There were four groups of rabbits: Three hour smoke-exposed rabbits (THSE, N=8), Three hour sham smoke-exposed rabbits (THSS, N=6), Short (3-4 min) smoke-exposed rabbits (SSE, N=5), and U75412E pretreated smoke-exposed (USE, N=7). The lungs were removed immediately after the experiment and bronchoalveolar lavage (BAL) was performed with normal sterile saline. BAL fluid was analyzed for vitamin E levels. The vitamin E concentrations (nmol/ mg protein in BAL fluid) were the following:

THSE	0.032 (0.004)
THSS	0.018 (0.001)
SSE	0.090 (0.029)*
USE	0.062 (0.005)*

* p < 0.05 -vs- THSE and THSS.

The USE rabbits lived for six hours after the smoke insult before they were killed for cell culture studies. Perhaps, the increased BAL vitamin E levels in the USE rabbits contributes to an attenuation of the smoke-induced lung injury by decreasing alveolar macrophage superoxide production. Supported by Upjohn and DoD Training Grant.

2363

PATHOLOGIC CHANGES AFTER JP-8 JET FUEL INHALATION IN FISCHER 344 RATS.
J Pfaff, G Parliman, K Parton, R. Lantz, H Chen, M Hays, M Witten. Steele Memorial Children's Research Center, Center for Toxicology, & Department of Pediatrics, Arizona Health Sciences Center, Tucson, AZ 85724.

Chronic exposure to the military jet fuel, JP-8, causes a variety of pulmonary symptoms. Inhalation studies with JP-8 have not identified a pulmonary lesion. We used one hour daily JP-8 aerosol exposures of Fischer 344 rats at concentrations of approximately 500 (495.15 - 519.85) and 950 (813 -1094) mg/m^3/hr. Subjects were exposed in a chamber with individual subject loading and nose-only presentation. For separate 7 and 28 day exposure groups we saw no pathologic lesion on light microscopy at the lower concentration. We observed pulmonary function changes in these groups for both compliance and resistance, as previously reported. With large dose exposures, not unrealistic on the flight-line, we demonstrate both lung function and pathologic change for both light and electron microscopy. Disruption of the alveolar-capillary membrane is observed in both epithelial and endothelial structures. Airways are convoluted and alveoli filled with red blood cells and fluid. We conclude that JP-8 alters pulmonary function through pathologic changes of lower respiratory structures.

Research supported by the U.S. Air Force, Office of Scientific Research, Grant# 91-0199.

DNA DAMAGE AND REPAIR (2364-2365)

2364

CELL CYCLE REGULATION OF A UNIQUE MULTIFUNCTIONAL DNA REPAIR GENE IN NORMAL HUMAN CELLS
Michael A. Sirover and Nasima Rahman Macaw. Fels Inst. for Cancer Res. and Molecular Biology and the Dept of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140

The cell cycle regulation of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)/uracil DNA glycosylase (UDG) gene was examined in normal human cells. Transcriptional expression was monitored by Northern blot analysis using a plasmid (pChug 20 [illegible]) which contained the 1.3 kb GAPDH/UDG cDNA. Translational regulation of the 37 kDa GAPDH/UDG protein was determined by the immunoprecipitation of the radiolabeled protein using an anti-human placental GAPDH/UDG monoclonal antibody. Steady state levels of the GAPDH/UDG mRNA was dependent on the cell cycle. A biphasic increase was observed resulting in a 19-fold increase as compared to that observed in G_o cells. A similar increase (7-fold) was observed for the biosynthesis of the 37 kDa GAPDH/UDG protein. A half life of less than 1 hr was detected for the newly synthesized 37 kDa protein by pulse chase experiments. DNA synthesis was maximal at 24 hr. At that interval GAPDH/UDG RNA and protein approached basal levels. These findings demonstrate the cell cycle regulation of the GAPDH/UDG gene in a defined relationship to the induction of DNA replication. The remarkably short half life of this reported multifunctional protein suggests its potential regulatory role in cell proliferation. In addition cell cycle regulation of the GAPDH/UDG gene demonstrates that it may not be used as a reported gene.

2365

A Ni^{2+}-BINDING PROTEIN IN *XENOPUS* OOCYTES AND EMBRYOS (*pNiXc*) HAS ALDOLASE ACTIVITY AND SEQUENCE HOMOLOGY TO HUMAN FRUCTOSE BISPHOSPHATE ALDOLASE A.
K. Antonijczuk, D.C. Henjum, A. Antonijczuk, A. Varghese, G. Korza, J. Ozols, S.M. Hopfer, and F.W. Sunderman Jr., Univ. of Conn. Med. School, Farmington, CT 06030.

$NiCl_2$ is teratogenic for *Xenopus laevis*, causing ocular, facial, cardiac, gut, and skeletal anomalies. Earlier studies detected three Ni^{2+}-binding proteins (*pNiXa*, 45 kD; *pNiXb*, 31 kD; *pNiXc*, 40 kD) in stage VI oocytes and embryos, based on autoradiograms of Western blots probed with $^{63}Ni^{2+}$. *In this study, pNiXc* was identified as an aldolase. After ovaries from mature *Xenopus* females were homogenized in tris buffer (pH 8) and centrifuged (5,400 g, 0.5 h; 40,000 g, 1 h), *pNiXc* was in the supernatant. The *pNiXc* stayed unbound following batch adsorption of other proteins on DEAE-cellulose; *pNiXc* was then adsorbed on cellulose phosphate and eluted in tris buffer with 0.25 M NaCl. The *pNiXc* was purified by HPLC (C-4 column, TFA/acetonitrile gradient), yielding a single protein band (mol. wt. 40 kD), based on SDS-PAGE analysis. After cleavage with CNBr or Lys C protease, eight peptides were prepared by HPLC and sequenced by Edman degradation, giving sequence data for 111 residues. Searches of the NBRF data-bank showed homology of *pNiXc* to many aldolases, including 82% sequence identity to human fructose bisphosphate aldolase A. Nondenatured *pNiXc*, isolated by FPLC on Ni-IDA Sepharose, showed aldolase activity in enzymatic assays. Inhibition of aldolase activity may be a mechanism for Ni^{2+} teratogenesis and embryotoxicity. (Supported by NIH grant ES-05331 and Northeast Utilities.)

2121

ALTERATION IN ALVEOLAR MACROPHAGE FUNCTION FOLLOWING ACUTE SMOKE EXPOSURE. R.C. Lang, G.J. Chen, A.M. Hays, M. Vermeulen and M. Witten. Depts. of Anatomy and Pediatrics, Univ. of Arizona Health Sciences Center, Tucson, AZ 85724 and Harvard Medical School, Boston, MA 02119.

New Zealand white rabbits were exposed to 60 tidal volumes of a synthetic smoke generated by the combustion of diesel fuel and polycarbonate plastic. Following exposure, animals were either sacrificed immediately (short term, N=6) or were maintained on a ventilator until they expired (2.95 ± 0.40 hrs) from the smoke exposure (long term, N=8). A third group was given a sham smoke exposure and sacrificed after 3 hrs (control, N=6). Pulmonary alveolar macrophages (PAM) were lavaged and cultured for up to 24 hrs. PAM were assessed for their ability to produce superoxide anion (O_2^-) and tumor necrosis factor-alpha (TNF-α). Lavage fluid was also analyzed for the presence of TNF-α. PAM from long term animals had both increased basal and zymosan stimulated O_2^- production immediately after lavage. This elevated production persisted for at least 24 hrs in culture. O_2^- production in PAM from short term animals was not different than controls. Conversely, TNF-α production in PAM from long term animals was suppressed immediately following lavage and only returned to control levels after 24 hrs in culture. In contrast, TNF-α production from short term animals was significantly elevated over control values 2 hrs after lavage. While TNF-α levels in lavage fluid were elevated in long term animals, values were not significantly different from controls. We conclude that TNF-α production is stimulated in PAM immediately following smoke exposure while priming of PAM for O_2^- production is delayed. Sponsored by UpJohn Pharmaceutical Company and DoD Training Grant.

2122

LOSS OF VLA-4 EXPRESSION OCCURS WITH MONOCYTE DIFFERENTIATION INTO MACROPHAGES. J.E. McEvoy, L.J. Embree, J.M. Harlan, R.K. Albert. University of Washington Medical Center, Seattle, WA 98195

We have shown that, unlike blood monocytes, alveolar macrophages do not express the β1 integrin VLA-4. We hypothesized this loss of VLA-4 expression occurred as a result of monocyte differentiation into macrophages. The promyelocytic leukemia cell lines THP-1 and HL-60 were stimulated for 24 hours with Phorbol 12-Myristate 13-Acetate (PMA,50ng/ml), then cultured in RPMI + 10% FBS for up to 5 days. Cells were assayed daily for surface expression of VLA-4, VLA-5 and β1 using flow cytometry and mRNA levels of VLA-4 via Northern analysis. Unstimulated cells were nonadherent and expressed VLA-4, VLA-5 and β1. Within 24 hr the cells became adherent. Flow cytometry showed decreased VLA-4 expression by 48 hr and its absence at 96 hr. VLA-4 mRNA decreased within 24 hr and was nearly undetectable by 72 hr. Surface expression of VLA-5 and β1 were unchanged throughout all 5 days. Loss of VLA-4 may be important with regard to 1) marking monocyte differentiation into macrophages, 2) as a model for studying control of β1 integrin expression and 3) mechanisms of monocyte recruitment from the vascular space.

2123

Alteration of intracellular pH (pHi) and calcium ([Ca^{2+}]i) in guinea pig alveolar macrophages during phagocytosis. Q.S. Qu, M. Ryan, and L.C. Chen. (SPON:C. Nadziejko) Dept of Environmental Medicine, New York University Medical Center, Long Meadow Rd, Tuxedo, NY 10987

The role of [Ca^{2+}]i and pHi in phagocytic function of guinea pig alveolar macrophages (AMØ) was investigated by measuring phagocytic index (PI, the percentage of viable AMØ that ingested at least one latex particle), phagocytic capacity (PC, the percentage of actively phagocytizing AMØ that ingested four or more particles), pHi and [Ca^{2+}]i simultaneously in AMØ incubated with opsonized Latex beads (3 μm) at 0, 15, 30, 45, 60, and 90 minutes after incubation. Before exposed to Latex beads AMØ were loaded with SNARF-1 and Fluo-3 together for pHi and [Ca^{2+}]i measurement. The resting [Ca^{2+}]i and pHi of AMØ was 84.88 ± 12.84 nM and 7.26 ± 0.08 (mean ± SD; n= 10), respectively. During incubation period, pHi decreased continuously with a concurrent increase in [Ca^{2+}]i. The values of pHi and [Ca^{2+}]i measured at and after 60 minutes were significantly different from corresponding values measured when phagocytosis was first initiated ($p<0.05$). The changes of pHi during phagocytosis correlate, to some extent, with changes of [Ca^{2+}]i ($r = 0.64$ $p = 0.0008$). Furthermore, phagocytic function of AMØ was closely associated with pHi and [Ca^{2+}]i.

	PI		PC	
	r	p	r	p
pHi	0.9123	0.0001	0.8436	0.0001
[Ca^{2+}]i	0.6564	0.0042	0.7886	0.0015

Our data suggest that both pHi and [Ca^{2+}]i are important physiological parameters governing phagocytosis in AMØ of guinea pigs.

2124

PULMONARY INTERSTITIAL MACROPHAGES (IM) AND DENDRITIC CELLS (DC) COOPERATE TO PROCESS PARTICULATE ANTIGEN. J.L. Gong, K.M. McCarthy, L.J. Tkachuk, E.E. Schneeberger. Massachusetts General Hospital, Boston, MA 02114

Ia^+, FcR^- interstitial lung DC, obtained at 95-98% purity from Lewis rats, do not endocytose PKH26 labeled heat killed Listeria monocytogenes (HKL), as visualized by fluorescence microscopy. In the total absence of macrophages, DC present this antigen poorly to HKL-immune T-cells (9,571±1,356 cpm) (mean±SE). Ia^- lung IM, obtained from the same rat lungs, avidly phagocytose PKH26 labeled HKL, but by themselves do not present HKL to HKL-immune T-cells (135±17 cpm). In an antigen presentation assay utilizing PKH26 labeled HKL, addition of IM ($5x10^4$/well) to lung DC ($5x10^3$/well), at a ratio of 1:10, results in a 300% increase in [^{3}H]TdR uptake by HKL-immune T-cells (27,438±1,427 cpm). Addition of >10% IM to the total accessory cell population, however, produces a progressive inhibition of [^{3}H]TdR uptake of HKL-immune T-cells. Supplementation with 10-30% (v/v) of 0.22 um filtered medium, derived from Ia^- IM ($2.5x10^6$/ml) that phagocytosed HKL for 16 h, results in a graded increase in [^{3}H]TdR uptake of HKL-immune T-cells in the presence of DC. Supernatants derived from larger numbers of IM (>$1x10^7$/ml), at the same IM:HKL ratio, are inhibitory when >10% (v/v) is added. It is concluded that small numbers of IM cooperate with DC to process and present particulate antigen; at higher numbers, however, IM inhibit antigen presentation by DC. Supported by NIH grant HL36781

2125

PULMONARY SYSTEMIC HOST DEFENSE PHAGOCYTES IN THE RAT. R.J. Fete, T.E. Hutson and G.D. Nielsen. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Bacteremia/endotoxemia stimulates an increase in the pulmonary host-defense capacity. The current study tested the hypothesis that microbial stimulation of the lung's systemic host-defense capacity is mediated by microvascular accumulation of activated mononuclear phagocytes. Sixteen male Sprague-Dawley rats (314±7 g) were anesthetized (10 mg Ketaset/100 g), equal volumes of the microbial product glucan (2 mg/100 g; n=9) or saline (n=7) infused via the dorsal penis vein and the animals permitted to recover. The rats were reanesthetized 48 hours later, intubated, and a catheter placed in the carotid artery. A test particulate, monastral blue B (9 mg/ 100 g), was infused and, 10 minutes later, the lungs inflation fixed with 2.5% glutaraldehyde (1 ml/100 g). Five random tissue samples were collected from each lung and ten high magnification (1000 x magnification under oil) fields/sample were histologically evaluated (50 fields/lung) to define the number of monastral blue B containing phagocytes. The multi-lobular nucleus of the neutrophil was used to differentiate it from mononuclear phagocytes. The number of neutrophils per field were the same in saline (0.89±0.67) and glucan challenged (0.79±0.41) rats. The number of mononuclear phagocytes, however, significantly ($p < 0.05$) increased from 3.69±0.59 active cells/field in the saline challenged rats to 6.47±2.0 active phagocytes/field in the glucan challenged rats. The results are consistent with the hypothesis that mononuclear phagocytes are the primary systemic host-defense cells in the rat lung and that circulating microbial products stimulate an increased accumulation of these activated phagocytes in the lung microvasculature.

Support: American Heart Association, Ohio Affiliate - the studies plus Mr. Hutson's Undergraduate Student Summer Research fellowship; American Physiological Society - Mr. Fete's High School Teacher Summer Research Fellowship.

2126

CYTOKINE EXPRESSION BY MULTINUCLEATED ALVEOLAR MACROPHAGES INDUCED BY COLONY STIMULATING FACTORS. I. Lemaire and H. Yang. Dept. of Pharm., Fac. Med., University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

Multinucleated giant alveolar macrophages (MGAM) are consistently found in lavage fluid and lung sections of animals with granulomatous inflammation. However, the mechanisms involved in MGAM formation and the precise role of these cells are unknown. In the present study, we investigated the in vitro effects of granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) on rat alveolar macrophages (AM) cultured in Lab Tek chambers. Incubation with M-CSF (25-500 U/ml) caused within 3 days a significant increase in the number of MGAM with 3 or more nuclei that resemble those observed in vivo. M-CSF induced equally well morphologically different types of MGAM: a) MGAM with a round shape and 3-8 nuclei (Type 1), b) MGAM with irregular shape and 8-30 nuclei (Type 2). GM-CSF within the same dose range induced predominantly the formation of Type 2 MGAM. Interestingly, TNF expression was detected by immunocytochemistry in MGAM. Type 1 MGAM (80-90%) expressed high levels of cytoplasmic TNF whereas Type 2 (20-30%) expressed lower levels of TNF. These data indicate that M-CSF and GM-CSF may be involved in the formation of MGAM and that these cells are competent for TNF production (supported by MRC).